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THE HEAT RESISTANCE OF SPORES WITH SPECIAL REFERENCE TO THE SPORES OF B. BOTULINUS

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This study is an investigation of the heat resistance of the spores of B. botulinus and the effects of several variable conditions which are ultimately responsible for their death. The conditions reported are: the effect of hydrogen and hydroxyl ion concentrations on thermal resistance and the effects of numbers of spores, desiccation, spore age and the presence of sodium chloride. A number of other factors and the intimate nature of the process of killing by heat under different conditions, and the laws governing it, are being studied. In addition to the purely academic aspects of the problem which this work covers, it is designed to furnish adequate criteria for the practical problems of sterilization and to insure the safety of processed foods with respect to botulism.

THERMAL DEATH RATE OF DIFFERENT STRAINS

For the purpose of our subsequent work and in order that the results obtained might have a practical application, it was necessary to determine the thermal resistance of the spores of all the available strains and to devote our attention to the most resistant one. There were sixteen strains in our laboratory at the time this work was begun, all of which were studied.

Strain 1. From the Museum of Natural History, New York; original source unknown; old laboratory strain of low virulence.

Strain 2. From the College of Physicians and Surgeons, New York; original source unknown; low virulence.

Strain 3. From San Jose, Calif. Isolated by Dickson from the crop of a chicken that died after eating home-canned string beans. The same beans had caused the death of one person. The strain was virulent.

Strain 4. From Hillsboro, Ore. Isolated by Dickson from the gizzard of a chicken that died after eating home-canned corn. The same corn had poisoned one person and about 50 chickens. The strain was virulent.

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Strain 5. From Escondido, Calif. It was isolated by Dickson from home-canned string beans from the same lot that had caused botulism in 7 persons, 4 of whom died. The strain was moderately virulent when first isolated.

Strain 6. Obtained from the Mulford Laboratories and supposed to have been isolated from cheese; low virulence.

Strain 7. From Seattle, Wash. Isolated by Dickson from the crop or gizzard of a chicken that had died after eating home-canned asparagus. The same asparagus had caused the death of four persons. Moderately virulent.

Strain 8. From Berkeley, Calif. Isolated by Dickson from a jar of home-canned string beans which was from the same lot that had poisoned a number of chickens. The strain was highly virulent.

Strain 9. Isolated by Dickson from dead chickens that were supposed to have eaten canned apricots; highly virulent.

Strain 10. Isolated by Dickson from hogs that were killed by eating canned peas; highly virulent.

Strain 11. Obtained from the New York Health Dept. Laboratories. Isolated by Nevin from a sample of home made cottage cheese; highly virulent.

Strain 12. Isolated by Graham and associated with forage poisoning in horses. The strain was toxic when first obtained in 1918; it has since lost its toxin producing power.

Strain 13. Isolated by Graham and associated with forage poisoning in horses; virulent.

Strain 14. Isolated by Graham from corn silage associated with an outbreak of forage poisoning among mules in Kentucky; low virulence.

Strain 15. Isolated by Edmondson at the Bureau of Chemistry, United States Dept. of Agriculture, from asparagus salad which had caused the death of four persons at Boise, Ida., January, 1919; virulent.

Strain 16. Isolated by Orr at the Harvard Medical School from ripe olives supposed to have caused botulism in 7 persons at Alliance, Ohio, in August, 1919; virulent.

The medium used for spore development was a mixture of two parts of finely ground sheep brain in one part of water. This was sterilized in the autoclave and used without adjustment. Its final reaction was neutral. The number and age of the spores was kept constant until the effect of variations in these factors could be determined and adequate controls applied. Spore development in the brain medium is rapid and reaches a total of approximately 15 million per c c when spore development is complete. This emulsion, undiluted, was exposed in 6 by three quarter inch test tubes for varying periods of time at 4 different temperatures in a DeKhotinsky bath. All the tubes were sealed with the exception of the one set exposed at 100 C. A loopful of the heated material was inoculated into meat infusion glucose agar tubes which were finally layered with paraffin oil and incubated at 37.5 C. The tubes were examined daily over a period of 4 months and the results recorded.

Spores approximately one month old were used. It was impossible to make all the exposures with spores of exactly the same age without introducing an error due to using spores from different bottles for the various exposures. As will be shown subsequently, the difference in thermal resistance due to a difference in age of several days when the spore is about one month old is too slight to be appreciable.

The four temperatures used were 100 C., 105 C., 120 C. and 95 C. for the less resistant strains. These, however, represent the thermometer reading inside the oil bath, and due to the radiation from the exposed surface of the tubes is not the true temperature inside the tubes. The lag or period necessary to raise the inside of the tubes from room temperature to a constant temperature of 99 (with the bath at 100) was about 30 minutes. The lag in the case of the 105 degree exposure was 15 minutes and in that of the 120 degree exposure 8 minutes. It is evident from table 1 that in the latter case the organisms were destroyed during the period of lag when the temperature inside the tube was rising, and long before the bath temperature was reached.

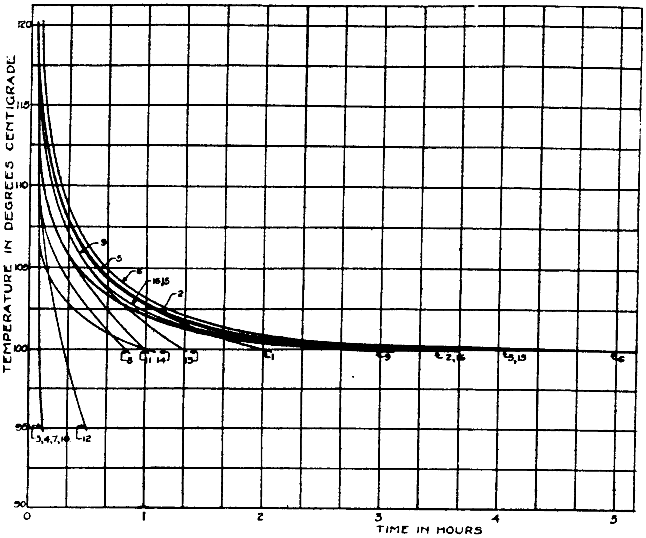

Chart 1.—Thermal death rate of spores of Bacillus botulinus.

Table 1 shows the results obtained using the 16 strains mentioned and chart 1 is a graphic representation of the same.

TABLE 1

Thermal Death Points of Different Strains of B. Botulinus

Strain Number	Bath Temperature																																	
	100 C.																			105 C.					120 C.		95 C.							
	Exposure in Minutes																			Exposure in Minutes					Exposure in Minutes		Exposure in Minutes							
	8	16	24	32	40	48	56	64	72	80	90	120	150	180	210	240	270	300	330	8	16	24	32	40	3	6	10	20	30	40	50	60	70	80
1	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	+	+	+	+	—	—	—								
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	+	+	+	+	—	—	—								
3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	+	+	+	+	—	—	—								
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	—	—	+	+	+	+	—	+	—								
7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—								
9	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	+	+	+	—	—	—	—								
10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
11	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—								
12	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	—	—	—	—	—
13	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	+	+	+	—	—	—	—	+	+	+	+	+	+	+	—
14	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	+	—	+	—	—	—	—								
15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	+	+	—	—	—	—	—								
16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	+	+	—	—	—	—	—								

It is evident that under the best conditions for survival the most resistant spores of B. botulinus will be destroyed within 5 hours at 100 C., within 40 minutes at 105 C., and within 6 minutes at 120 C.

The thermal resistance varied considerably with the different strains. Spores, which were in apparently no other way dissimilar, showed marked differences in this respect, and the extent of the variation is well illustrated by the 100 C. exposure. At that temperature 4 strains were killed in 8 minutes, while it required from 3 to 5 hours at the same temperature to destroy the 6 that were most resistant. We have not attempted to correlate this variation with others that the strains may have exhibited. But it was evident that spore resistance is independent of toxin development, and that both toxic and atoxic strains may be either resistant or nonresistant. It was observed, however, that the most resistant strains sporulated most actively.

In a similar investigation, using 10 strains of B. botulinus, Burke[1] recently obtained results which are for the most part similar to our own. She, however, found one strain (X) which survived an autoclaving at 15 lbs. (121.3 C.) for 10 minutes and at 10 lbs. (115.6 C.) for 20 minutes. This same strain did not survive an autoclaving at 5 lbs. (108.8 C.) for 20 minutes. On the basis of this, Burke concludes that autoclaving at 15 lbs. (121.3 C.) for 10 minutes is insufficient to destroy the spores of B. botulinus. My results indicate that all spores of B. botulinus will be killed within 6 minutes at a bath temperature of 120 C. and that the greater number of them will be destroyed within 3 minutes. The results obtained by Burke may be explained by the fact that she used only pressure gage readings as an index of the temperature in the autoclave, and that there was apparently no adequate control of the true temperature inside the tubes.

EFFECT OF AGE AND NUMBER OF SPORES ON THE THERMAL DEATH RATE

In this part of the investigation and for all the subsequent work we used the spores of a single strain, 15, and varied one factor, keeping all the others constant. This strain was chosen from among the 16 strains because it best satisfied the conditions we applied. It came from an outbreak which was unquestionably botulism; it is, with the exception of strain 6, the most resistant; and it is an active gas producer on glucose mediums, forming spores readily and producing toxin.

Technic.—The technic was standardized and adhered to in all the various tests which follow. Bigelow and Esty[2] have described a technic somewhat similar to our own but differing from it in several essential respects. Special tubes, 10 mm. inside diameter, 12 mm. outside diameter, and 12 inches long, were used. These were inoculated with the spore emulsion previously filtered through a sterile gauze filter to remove the large particles. The sides of the tubes were then thoroughly heated to within an inch of the top of the fluid. When these had cooled they were sealed in the flame and exposed in a de Khotinsky bath. A series was prepared in this manner and at the end of each interval of time one tube was removed, opened and glucose agar added, care being exercised to mix thoroughly the spore emulsion throughout the agar. If the spores had been in either acid or alkali, the tubes would be neutralized with equal parts of sterile and previously standardized hydrochloric acid or sodium hydroxide. Thus for each dilution of acid or alkali in which the spores were heated a sterile solution was used that would neutralize it in equal volume.

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[1] Jour. Am. Med. Assn., 1919, 72, p. 88.

[2] Abstracts of Bact., 1920, 4, p. 10.

The glucose agar tubes were then layered with paraffin oil and incubated at 37.5 C. for at least 4 months.

There is evidently a gradual destruction of the spore, not an instantaneous killing (table 3). As the heating continues the spore appears to be gradually weakened. This is evident from the rate of development after exposure. As a general rule, the longer the period of exposure at any temperature, the longer the time required for the spore to vegetate until finally the injury is sufficient to destroy it. In one case growth did not appear until 91 days after exposure. This phase of the thermal death rate will be treated more fully in another paper.

In determining the effect of age on spore resistance several bottles of the same brain medium were inoculated at intervals from a 24-hour culture of B. botulinus and incubated. Four spore emulsions were used, respectively 34, 56, 122 and 163 days old. These were adjusted with 0.5% salt solution to 1,500,000 spores per c c, and tubes containing 1 c c were exposed at 98 C.

The 163-day spore survived for 30 minutes, but was killed in 60 minutes. The other 3 survived after 120 minutes heating, but were killed in 150 minutes. Of these, the 34-day spore showed the least retardation in vegetation, coming up on the fifth day. The 56-day old spore did not vegetate until the 28th day and the 122-day old spore did not vegetate until the 50th day.

TABLE 2

EFFECT OF AGE ON THERMAL RESISTANCE

Age of Spore in Days	Result of Heating at 98 C.	Retardation in Development of Last Surviving Spore
34	Survives 120 minutes; killed in 150 minutes	5 days
56	Survives 120 minutes; killed in 150 minutes	28 days
122	Survives 120 minutes; killed in 150 minutes	50 days
163	Survives 30 minutes; killed in 60 minutes	

It is evident from this that the oldest moist spore is the most severely injured during heating, and that the 34-day spore is the most resistant. This is contrary to the general conception that the resistance of a spore increases with age. This belief may have arisen from the fact that the aging of a spore is usually accompanied by drying, and it is this drying, not the aging, that is responsible. We have in progress investigations on the effect of drying on resistance to heat. This indicates that injury and death are the result of protein coagulation, and that the decrease of the moisture content of the spore decreases the effectiveness of the heating. If it were true that the resistance of a spore increased with age, logical reasoning would lead us to conclude that the spore cell is in itself immortal.

Another test was made combining both the effect of age and the effect of numbers of spores. Eighty and 18-day spores and concentrations of 1,500,000 and 15,000,000 per c c of each were used. The exposure temperature was 100 C. The results are given in table 3.

TABLE 3

EFFECT OF AGE AND DILUTION ON THERMAL RESISTANCE

	Exposure in Minutes at 100 C.							
	0	30	60	90	120	150	180	210
18-day spore, 15,000,000 per c c	1	2	4	9	16	30		
18-day spore, 1,500,000 per c c	1	4	5	17				
80-day spore, 15,000,000 per c c	1	5	29					
80 day spore, 1,500,000 per c c	1	5	7					

The numbers indicate the incubation period in days before growth appeared.

As in the former case, the younger spore was found to be more resistant in both suspensions.

Regarding the effect of numbers, it is evident in the case of the younger spores, both from the period of survival and the greater lag in the more dilute emulsion at any one period, that the greater the concentration the greater the resistance. The theoretical consideration which this gives rise to will be offered in a later paper.

In the case of the 80-day spore it appears that dilution does not have such a pronounced effect. This indicates that as a culture ages there is a general retrogression, but that the more resistant individuals change more rapidly than the others with a resulting tendency toward stabilization, the ultimate resistance of the individual more nearly approximating the average resistance.

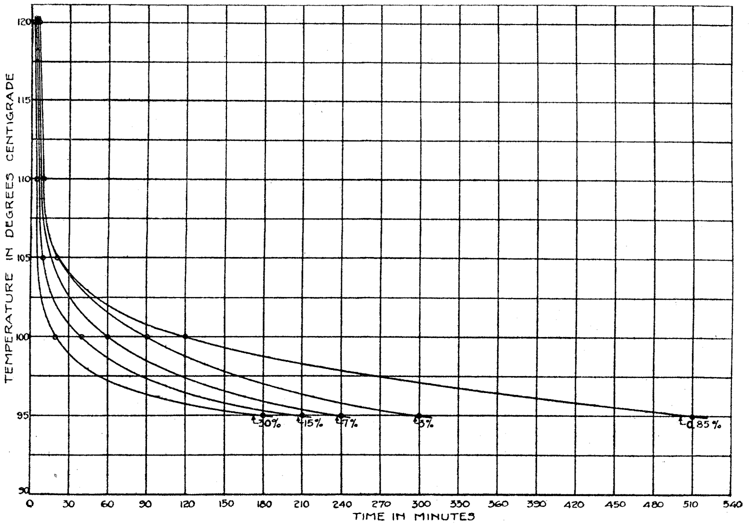

Chart 2.—Thermal death rate of spores of Bacillus botulinus in different concentrations of sodium chloride.

EFFECT OF SALT ON THERMAL DEATH RATE

The spores of strain 15 were exposed in 5 different strengths of sodium chloride at 5 different temperatures. The hydrogen-ion concentration was kept practically constant, that is, within limits that would have no practical effect. Ten parts of the salt solution were mixed with one part of the filtered spore emulsion and distributed so that each tube exposed contained 1,500,000 spores in 1.1 c c volume. In this, as in all other work in which dilutions were used, the molar strength was made the basis of comparison.

The results are given in table 4 and are represented graphically in chart 2.

TABLE 4

SODIUM CHLORIDE

Strength		$[\overset{+}{\mathrm{H}}]$	pH	Exposure in Minutes																																			
In Molar Solution	Per Cent.			At 95 C.																		At 100 C.									At 105 C.			At 110 C.			At 120 C.		
				0	30	60	90	120	150	180	210	240	270	300	330	360	390	420	450	480	510	0	10	20	30	40	50	60	90	120	0	10	20	0	5	10	0	3	6
5 M	30	8.0×10^{-8}	7.1	+	+	+	+	+	+	—	—	—	—	—	—	..	..	..	..	..	..	+	+	—	—	—	—	—	—	—	+	—	—	+	—	—	+	—	—
2.5 M	15	4.5×10^{-8}	7.35	+	+	+	+	+	+	+	—	—	—	—	—	—	..	..	..	..	..	+	+	+	+	—	—	—	—	—	+	—	—	+	+	—	+	—	—
1.2 M	7	3.5×10^{-8}	7.46	+	+	+	+	+	+	+	+	—	—	—	—	—	—	..	..	..	..	+	+	+	+	+	+	—	—	—	+	+	—	+	+	—	+	+	—
0.5 M	3	4.6×10^{-8}	7.34	+	+	+	+	+	+	+	+	—	+	—	—	—	—	—	..	..	..	+	+	+	+	+	+	+	—	—	+	+	—	+	+	—	+	+	—
0.14 M	0.85	4.6×10^{-8}	7.34	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	—	+	+	+	+	+	+	+	+	—	+	+	—	+	+	—	+	+	—

+ indicates growth; — indicates no growth.

TABLE 5

HYDROCHLORIC ACID

Strength		$[\overset{+}{\mathrm{H}}]$	pH	Exposure in Minutes																									
In Molar Solution	Per Cent.			At 95 C.												At 100 C.					At 105 C.			At 110 C.			At 120 C.		
				0	30	60	90	120	150	180	210	240	270	300	330	0	10	20	30	40	0	10	20	0	5	10	0	3	6
0.2 M	0.7	4.5×10^{-2}	1.35	+	—	—	—	—	—	—	—	—	—	—	—	+	+	—	—	—	+	—	—	+	—	—	+	—	—
0.1 M	0.35	1.6×10^{-2}	1.80	+	+	—	—	—	—	—	—	—	—	—	—	+	+	—	—	—	+	—	—	+	—	—	+	—	—
0.05 M	0.18	1.5×10^{-3}	2.82	+	+	+	—	—	—	—	—	—	—	—	—	+	+	+	+	—	+	—	—	+	+	—	+	—	—
0.01 M	0.04	1.9×10^{-6}	5.72	+	+	+	+	+	+	+	+	+	+	+	—	+	+	+	+	—	+	+	—	+	+	—	+	+	—

+ indicates growth; — indicates no growth.

The 5 temperatures of exposure used in this, as in all the subsequent work, were 95 C., 100 C., 105 C., 110 C. and 120 C. These represent the bath temperatures and are given in the tables. The liquid inside the tubes does not reach the bath temperature until several minutes after they had been exposed. The period necessary to raise the temperature will vary with the particular technic employed. In our case it was 8 to 12 minutes. It is evident from this, therefore, that at exposures of 120 C., in which killing occurs in 6 minutes or less, the actual thermal death point of the organism is several degrees lower.

To what particular factor or to what combination of factors the toxicity of sodium chloride is due, we are as yet unable to conclude. The various possibilities that present themselves are the action of the free sodium and chlorine ions, the undissociated salt, and the physical factors such as change in osmotic pressure and the presence of a large amount of electrolyte. It is also doubtful just what the mechanism of the action is, whether it is a direct intoxication in the sense of a chemical poisoning or what appears more probable, an increase in the coagulability of the cell protein.

EFFECT OF H AND OH ION ON THERMAL DEATH RATE

In determining the effect of the hydrogen and hydroxyl ion concentration on the thermal death rate, the technic previously described was used. Hydrochloric acid, oxalic acid, tartaric acid, lactic acid, acetic acid and citric acid were used. One alkali, sodium hydroxide, was also used. Each of these was made up in 4 dilutions on the basis of molar strength, and their effect was determined on measured numbers of the free spore at 5 different temperatures ranging from 95 C to 120 C.

Ten parts of the solution were mixed with one part of spore emulsion so that each tube contained 1,500,000 spores in 1.1 c c of volume. The hydrogen-ion concentration of the mixture was determined electrometrically. After exposure the tubes were neutralized and 10 c c of 1% glucose agar added. They were then layered with paraffin oil and incubated for at least 4 months at 37.5 C.

Tables 5 to 10 inclusive give the results obtained with 4 dilutions of each of the 6 acids previously mentioned. The dilutions of each acid were made to cover a hydrogen-ion range from a point close to neutrality to the practical limit of its dissociation with the spore emulsion present. Charts 3 to 8 inclusive are graphic representations of the same.

TABLE 6

OXALIC ACID

Strength		$\overset{+}{[H]}$	p_H	Exposure in Minutes																												
In Molar Solution	Per Cent.			At 95 C.														At 100 C.						At 105 C.			At 110 C.			At 120 C.		
				0	30	60	90	120	150	180	210	240	270	300	330	360	390	0	10	20	30	40	50	0	10	20	0	5	10	0	3	6
0.1 M	1.3	1.0×10^{-2}	2.0	+	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	+	—	—	+	—	—	+	—	—
0.05 M	0.63	2.0×10^{-3}	2.70	+	+	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	—	—	—	+	—	—	+	—	—	+	—	—
0.01 M	0.13	2.2×10^{-5}	4.66	+	+	+	+	+	—	—	—	—	—	—	—	—	—	+	+	+	+	—	—	+	—	—	+	+	—	+	—	—
0.001 M	0.013	4.5×10^{-8}	7.35	+	+	+	+	+	+	+	+	+	—	+	—	+	—	+	+	+	+	+	—	+	—	—	+	+	—	+	—	—

+ indicates growth; — indicates no growth.

TABLE 7

TARTARIC ACID

Strength		$\overset{+}{[H]}$	p_H	Exposure in Minutes																																		
In Molar Solution	Per Cent.			At 95 C.																	At 100 C.									At 105 C.			At 110 C.			At 120 C.		
				0	30	60	90	120	150	180	210	240	270	300	330	360	390	420	450	480	0	10	20	30	40	50	60	90	120	0	10	20	0	5	10	0	3	6
0.1 M	1.5	9.5×10^{-4}	3.03	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	—	—	—	—	—	—	+	+	—	+	—	—	+	—	—
0.05 M	0.75	4.0×10^{-4}	3.40	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	—	—	—	—	—	—	+	—	—	+	—	—	+	—	—
0.01 M	0.15	1.5×10^{-5}	4.82	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—	—	+	—	—	+	—	—	+	—	—
0.001 M	0.015	1.3×10^{-7}	6.89	+	+	+	+	+	+	+	+	+	+	+	+	—	+	—	+	—	+	+	+	+	+	+	+	+	—	+	—	—	+	+	—	+	—	—

+ indicates growth; — indicates no growth.

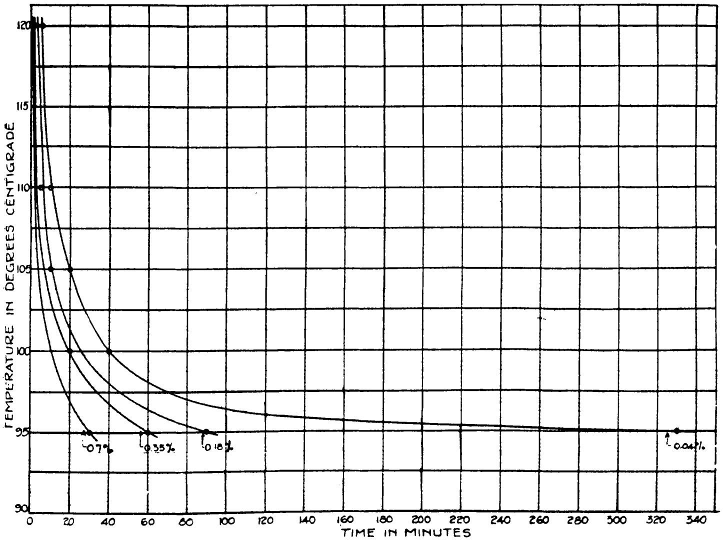

Chart 3.—Thermal death rate of spores of Bacillus botulinus in hydrochloric acid.

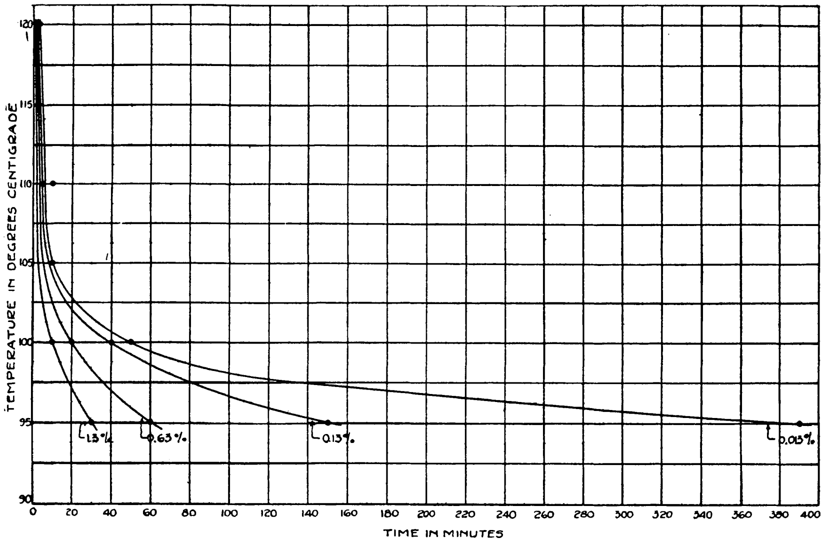

Chart 4.—Thermal death rate of spores of Bacillus botulinus in oxalic acid.

TABLE 8

LACTIC ACID

Strength		$\overset{+}{[H]}$	pH	Exposure in Minutes																						
In Molar Solution	Per Cent.			At 95 C.									At 100 C.					At 105 C.			At 110 C.			At 120 C.		
				0	30	60	90	120	150	180	210	240	0	10	20	30	40	0	10	20	0	5	10	0	3	6
0.2 M	1.8	4.0×10⁻⁴	3.40	+	+	—	—	—	—	—	—	—	+	+	—	—	—	+	—	—	+	—	—	+	—	—
0.1 M	0.9	3.5×10⁻⁴	3.46	+	+	+	—	—	—	—	—	—	+	+	+	—	—	+	—	—	+	+	—	+	—	—
0.05 M	0.45	2.6×10⁻⁴	3.59	+	+	+	+	+	+	—	—	—	+	+	+	+	—	+	—	—	+	+	—	+	—	—
0.01 M	0.09	1.0×10⁻⁵	5.00	+	+	+	+	+	+	+	+	—	+	+	+	+	—	+	—	—	+	+	—	+	—	—

+ indicates growth; — indicates no growth.

TABLE 9

ACETIC ACID

Strength		$\overset{+}{[H]}$	pH	Exposure in Minutes																															
In Molar Solution	Per Cent.			At 95 C.																	At 100 C.						At 105 C.			At 110 C.			At 120 C.		
				0	30	60	90	120	150	180	210	240	270	300	330	360	390	420	450	480	0	10	20	30	40	50	0	10	20	0	5	10	0	3	6
0.2 M	1.2	6.5×10⁻⁵	4.19	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	..	+	+	+	—	—	—	+	+	—	+	+	—	+	—	—
0.1 M	0.6	3.5×10⁻⁵	4.46	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	..	+	+	+	+	—	—	+	+	—	+	+	—	+	+	—
0.05 M	0.3	1.5×10⁻⁵	4.82	+	+	+	+	+	+	+	—	+	—	—	—	—	—	—	—	..	+	+	+	+	—	—	+	+	—	+	+	—	+	—	—
0.01 M	0.06	9.0×10⁻⁷	6.05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	+	+	+	—	+	+	—	+	+	—	+	—	—

+ indicates growth; — indicates no growth.

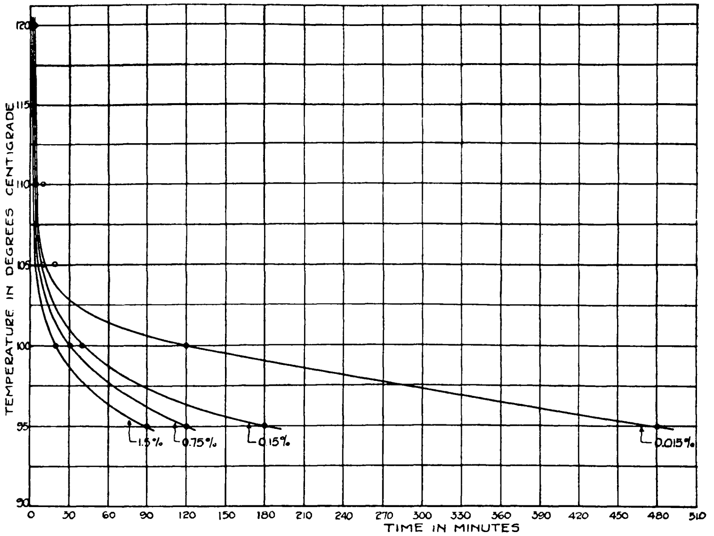

Chart 5.—Thermal death rate of spores of Bacillus botulinus in tartaric acid.

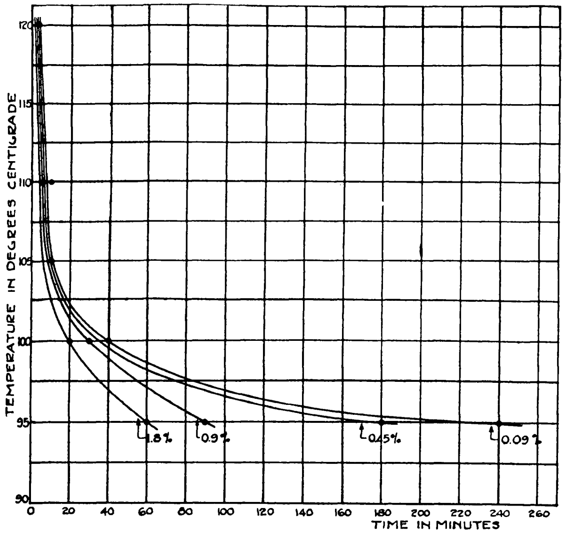

Chart 6.—Thermal death rate of spores of Bacillus botulinus in lactic acid.

TABLE 10

Citric Acid

Strength		$[\overset{+}{H}]$	pH	Exposure in Minutes																															
In Molar Solution	Per Cent.			At 95 C.															At 100 C.								At 105 C.			At 110 C.			At 120 C.		
				0	30	60	90	120	150	180	210	240	270	300	330	360	390	420	0	10	20	30	40	50	60	90	0	10	20	0	5	10	0	3	6
0.1 M	2.1	7.0×10^{-4}	3.16	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	+	—	—	+	—	—	+	—	—
0.05 M	1.05	3.5×10^{-4}	3.46	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—	+	—	—	+	+	—	+	—	—
0.01 M	0.2	1.3×10^{-5}	4.89	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	+	—	—	+	+	—	+	—	—
0.001 M	0.02	2.2×10^{-7}	6.66	+	+	+	+	+	+	+	+	+	+	+	—	+	+	—	+	+	+	+	+	+	+	—	+	—	—	+	—	—	+	—	—

+ indicates growth; — indicates no growth.

TABLE 11

Sodium Hydroxide

Strength		$[\overset{+}{H}]$	pH	Exposure in Minutes																																
In Molar Solution	Per Cent.			At 95 C.																	At 100 C.							At 105 C.			At 110 C.			At 120 C.		
				0	30	60	90	120	150	180	210	240	270	300	330	360	390	420	450	480	0	10	20	30	40	50	60	0	10	20	0	5	10	0	3	6
0.2 M	0.8	3.8×10^{-12}	11.42	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	—	—	—	—	+	—	—	+	—	—	+	—	—
0.1 M	0.4	7.5×10^{-11}	10.13	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	—	—	—	—	+	—	—	+	—	—	+	—	—
0.05 M	0.2	5.5×10^{-10}	9.26	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	+	+	+	+	—	—	—	+	+	—	+	+	—	+	—	—
0.01 M	0.04	6.0×10^{-9}	8.22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	+	+	+	+	—	+	+	—	+	+	—	+	—	—

+ indicates growth; — indicates no growth.

Oxalic acid is the only acid in which the negative ion appeared to exert any toxic effect. In the very high dilution, in this case 0.001 molar, the emulsion is alkaline due to the fact that the spore emulsion with which the acid is mixed becomes alkaline during the growth of the organism. This change shall be taken up separately in another place.

Since for the practical purposes of our problem we were interested more particularly in the acid range, only one alkali was used to determine the effect of hydroxyl ion, and to cover the range of hydrogen-ion concentration from a maximum to a minimum. Four dilutions of sodium hydroxid, 0.2 molar, 0.1 molar, 0.05 molar and 0.01 molar were used. The results are shown in table 11 and are graphically represented in chart 11.

In order to determine the general effect of hydrogen ion on thermal death rate the thermal death points at one temperature were plotted with P_H as the abscissa and time necessary to produce death as the ordinate. Two such curves are given, one for a temperature of 95 C. (chart 10) and the other for 100 C. (chart 11). At the higher temperatures the killing is too rapid to make such curves of any value.

It is evident from the accompanying charts that the greatest resistance to killing by heat occurs close to the neutral point and that as the hydrogen-ion concentration increases it is accompanied by a rapid reduction in that resistance. It is, furthermore, apparent that the most pronounced change in resistance occurs just beyond the neutral point and that as the hydrogen-ion concentration increases further the decrease in resistance for a similar change in hydrogen-ion concentration becomes less. In other words, the rate of loss of resistance decreases as the hydrogen-ion concentration increases.

Comparing the two charts, we can also conclude that this change in rate with change in hydrogen-ion concentration becomes more pronounced as the temperature increases.

Regarding the alkaline side of the curve, it appears that the hydroxyl ion has nearly, if not exactly, the same effectiveness as the hydrogen ion.

PRACTICAL APPLICATION

In order that the practical application of these results might be facilitated charts 12 and 13 were prepared. Chart 12 gives the thermal death point for the spores of B botulinus under the conditions of our

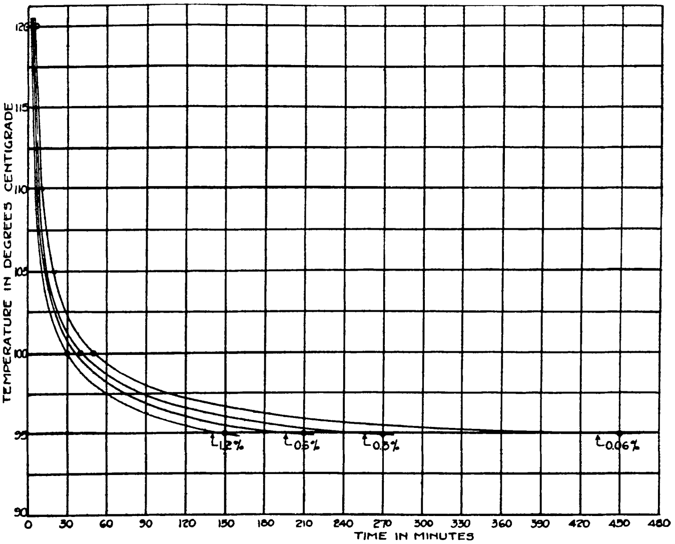

Chart 7.—Thermal death rate of spores of Bacillus botulinus in acetic acid.

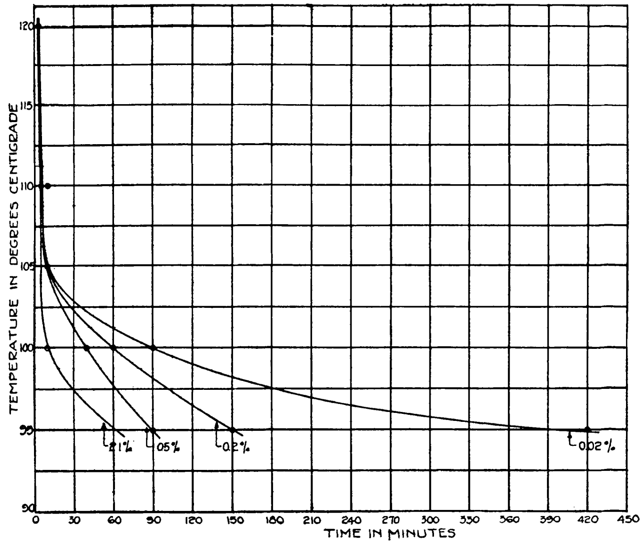

Chart 8.—Thermal death rate of spores of Bacillus botulinus in citric acid.

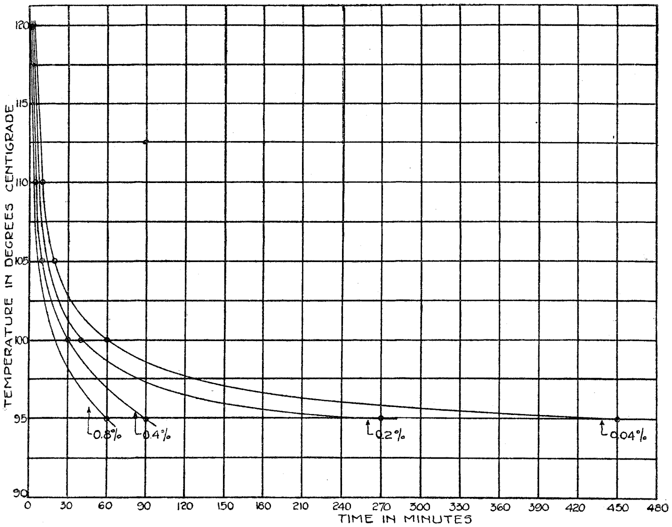

Chart 9.—Thermal death rate of spores of B. botulinus in sodium hydroxide.

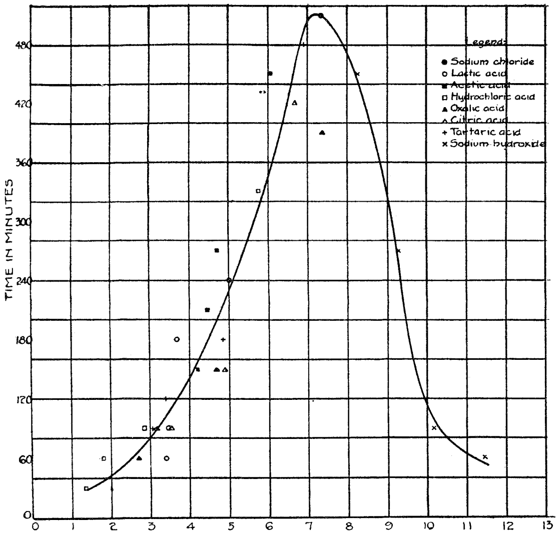

Chart 10—Change in the thermal death point at 95 C. of spores of B. botulinus with change in PH value.

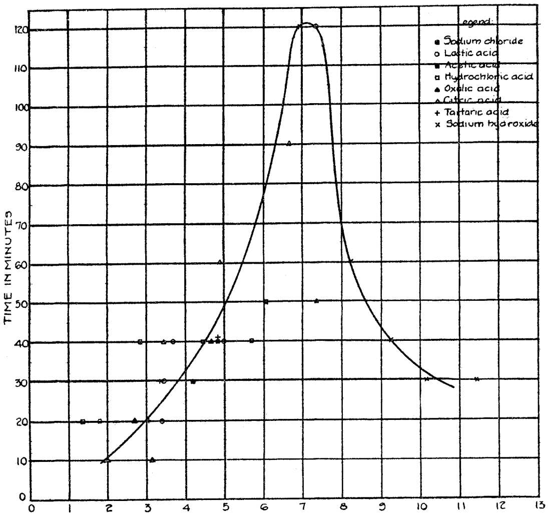

Chart 11.—Change in the thermal death point at 100 C. of spores of B. botulinus with change in PH value.

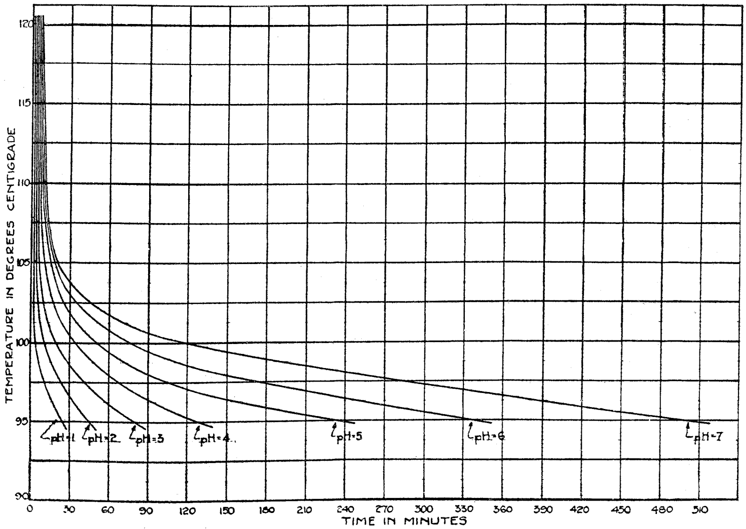

Chart 12.—Thermal death rate of spores of B. botulinus at PH values 1 to 7, inclusive

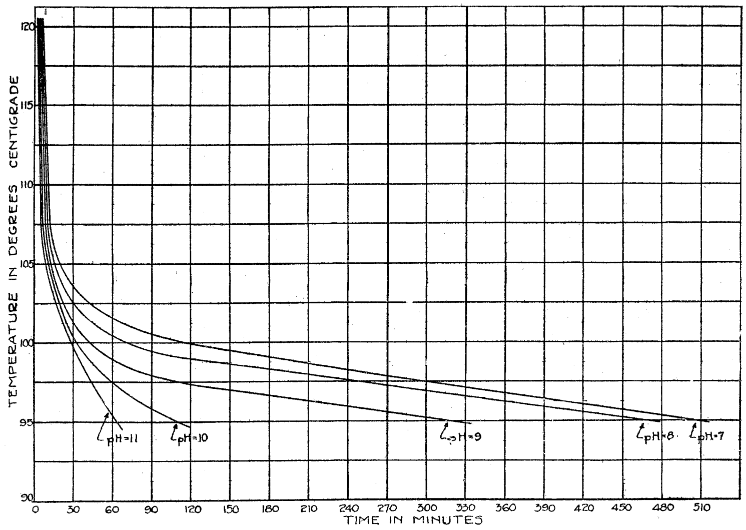

Chart 13.—Thermal death rate of spores of B. botulinus at Pн values 7 to 11, inclusive.

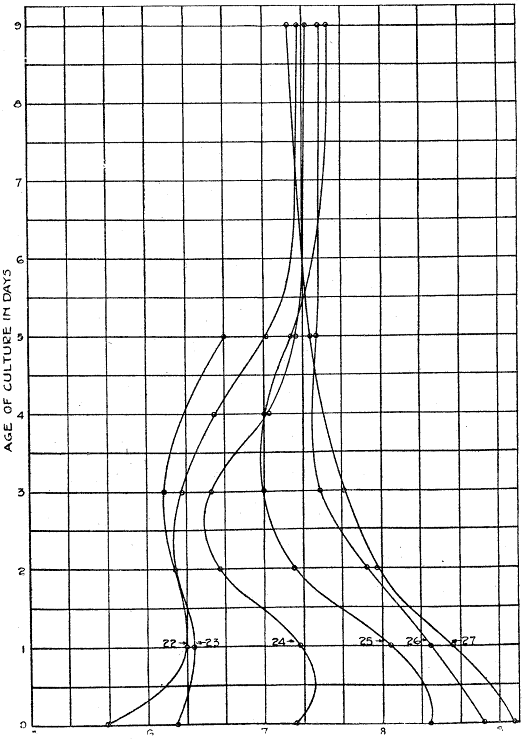

Chart 14.—Change in hydrogen-ion concentration during growth of B. botulinus.

TABLE 12

CHANGE IN HYDROGEN-ION CONCENTRATION IN SIX BOTTLES CONTAINING COAGULABLE MEAT PROTEIN AND 1% GLUCOSE

Medium No.	Uninoculated		1 Day		2 Days		3 Days		4 Days		5 Days		9 Days	
	$\overset{+}{H}$	p_H	$\overset{+}{H}$	p_H	$\overset{+}{H}$	p_H	$\overset{+}{H}$	p_H	$\overset{+}{H}$	p_H	$\overset{+}{H}$	p_H	$\overset{+}{H}$	p_H
22	2.3×10^{-6}	5.64	4.6×10^{-7}	6.34	6×10^{-7}	6.22	7.5×10^{-7}	6.13	....	....	2.2×10^{-7}	6.66		
23	6×10^{-7}	6.22	4×10^{-7}	6.40	5.8×10^{-7}	6.24	5×10^{-7}	6.30	2.7×10^{-7}	6.57	9.5×10^{-8}	7.03	5×10^{-8}	7.30
24	5.6×10^{-8}	7.25	5.3×10^{-8}	7.28	2.4×10^{-7}	6.62	2.8×10^{-7}	6.55	$1.0\times10^{7-}$	7.00	5.8×10^{-8}	7.24	4.5×10^{-8}	7.38
25	4×10^{-9}	8.40	8.5×10^{-9}	8.07	6×10^{-8}	7.22	1.0×10^{-7}	7.00	9.5×10^{-8}	7.03	6×10^{-8}	7.22	2.9×10^{-3}	7.54
26	1.4×10^{-9}	8.85	4×10^{-9}	8.40	1.3×10^{-8}	7.89	3.3×10^{-8}	7.48	....	....	3.5×10^{-8}	7.46	3.3×10^{-8}	7.48
27	7.5×10^{-10}	9.13	2.5×10^{-9}	8.60	1.1×10^{-8}	7.96	2.1×10^{-8}	7.68	....	....	4.0×10^{-8}	7.40	6.5×10^{-8}	7.19

experiments at any temperature between 95 and 120 and at any P_H value in the acid range. Chart 13 gives the same for the alkaline range.

The P_H value must be determined immediately before the exposure is made. Should the spores of B. botulinus grow in a favorable medium the P_H value will change rapidly and ultimately reach the hydrogen-ion concentration at which it is most difficult to produce killing, that is, close to the neutral point.

In order to determine this effect, 6 bottles of meat infusion agar containing coagulable meat protein and 1% glucose were set at different hydrogen-ion concentrations and inoculated. The change in hydrogen-ion concentration was noted over a period of 9 days, when it became practically stable.

The changes are given in table 12 and are plotted in chart 14.

From these results it can be seen how essential it is to determine the P_H value just before sterilization on account of the rapidity of change and also since the general tendency is toward the value when a longer period of exposure is required.

Furthermore, for the practical purposes of canning, the heat penetration into the particular container used should be determined, and a sufficient factor of safety allowed.

SUMMARY AND CONCLUSIONS

The free spores of B. botulinus are destroyed within 5 hours at 100 C., within 40 minutes at 105 C., and within 6 minutes at 120 C. Bath temperatures are indicated. These thermal death points were determined under optimum conditions for survival.

The destruction of the spore is a gradual process, not an instantaneous killing and is probably due to a gradual protein coagulation. The spores are evidently injured before they are killed. This is inferred from the fact that the more protracted the period of heating, before killing occurs, the longer the period required for the spore to vegetate.

Young moist spores have a higher thermal resistance than old moist spores. Spores that are 1 month old are found to be three times as resistant as spores that are 5 months old.

There is a general decrease in thermal resistance as the spore ages. The more resistant individuals change more rapidly than the less resistant ones causing a tendency toward stabilization, the ultimate resistance of the individual more nearly approximating the average resistance. This is shown by the effect of dilution on the thermal resistance of young and old spores, the old spore emulsions being practically unaffected by changes in the number of spores present, while the young spore emulsions show marked decrease in resistance as the dilution increases.

The thermal resistance of emulsions of young spores increases as the concentration of the emulsion increases.

Sodium chloride considerably lowers the thermal resistance and the rate of this lowering increases rapidly as the concentration of the salt is increased.

The hydrogen ion lowers the thermal resistance of the spore and the rate of this reduction decreases as the hydrogen-ion concentration increases.

The hydroxyl ion lowers the thermal resistance and the rate of the reduction decreases as the hydroxyl-ion concentration increases.

The hydrogen-ion concentration changes considerably in a medium in which B. botulinus is growing and ultimately stabilizes itself at a point near a P_H value of 7.5.

In applying these results to the practical problems of processing canned foods, it is necessary to determine the P_H value of the material to be sterilized immediately before the exposure. Any delay between the determination and the processing may cause a sufficient change in the P_H value to require a higher temperature or a longer period of exposure.

In all practical processing methods a sufficient safety factor should be allowed. The actual time required in applying such a factor becomes rapidly less as the temperature of processing is increased. Thus, a 50% safety factor applied at a processing temperature of 100 C., the medium to be sterilized having a P_H value of 7, would require an extra heating of 60 minutes or a total of 180 minutes. The same safety factor applied at a processing temperature of 120 C., the medium to be sterilized being the same, would require an extra heating of 3 minutes or a total of 9 minutes.